



# **Corticosterone Enzyme Immunoassay Kit**

## **User Manual**

Catalog#	K3014-1	1 Plate Kit
	K3014-5	5 Plate Kit

# TABLE OF CONTENTS

Intended Use	3
Background	3
Assay Principle	3
Kit Components	4
Materials Required	4
Precautions	4
Reagent Preparation	5
Normal Range Standards	5
Expanded Range Standards	6
Sample Preparation	7
Assay Protocol	8
Calculations	9
Typical Standard Curve: Example	9

## Notice to Purchaser

**This product is to be used for Research Purposes Only. It is not to be used for Drug or Diagnostic Purposes, nor is it intended for Human Use. B-Bridge products may not be resold, modified for resale, or used to manufacture commercial products without the express written consent of B-Bridge International, Inc.**

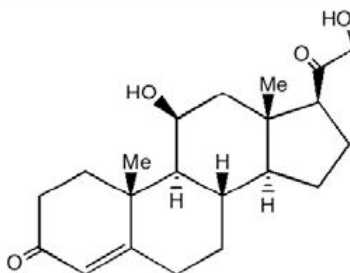
EXCEPT AS OTHERWISE EXPRESSLY SET FORTH IN THIS USER MANUAL, B-BRIDGE DOES NOT MAKE ANY REPRESENTATION OR WARRANTIES OR CONDITIONS OF ANY KIND, EITHER EXPRESSED OR IMPLIED, WITH RESPECT TO THE PRODUCTS, OR INFORMATION DISCLOSED HEREUNDER, INCLUDING, BUT NOT LIMITED TO, THE IMPLIED WARRANTIES OF MERCHANTABILITY, FIT FOR A PARTICULAR PURPOSE, OR NONINFRINGEMENT OF THE INTELLECTUAL PROPERTY RIGHTS OF THIRD PARTIES.

## INTENDED USE

The B-Bridge Corticosterone ELISA Kit quantitatively measures Corticosterone present in dried fecal extracts, serum, EDTA and heparin plasma samples, and tissue culture medium. Please read the complete kit insert before performing this assay. This kit is species independent.

## BACKGROUND

Corticosterone is a glucocorticoid secreted by the cortex of the adrenal gland and is produced in response to stimulation of the adrenal cortex by ACTH and the precursor of aldosterone. Corticosterone is a major stress steroid. Since the chemical structure of corticosterone appears identical across all known species, the assay provides consistent results regardless of the species source of the samples.



Corticosterone

## ASSAY PRINCIPLE

The B-Bridge Corticosterone ELISA Kit uses an antibody sandwich to link corticosterone to the microtiter plate. Levels of corticosterone are measured by competition with known amounts of a detectable corticosterone-peroxidase conjugate. The procedure is straightforward and involves only two incubations: a 1 hour incubation to capture the corticosterone complexes followed by a second 30 minute incubation to develop the detection reagent.

The microtiter plate has been coated with antibodies that recognize sheep antibodies. Sheep anti-corticosterone antibodies are then used to capture corticosterone and bind it to the plate. A corticosterone level in the samples is quantified using a corticosterone-peroxidase conjugate. Corticosterone in the samples competes with the corticosterone-peroxidase conjugate. After the reaction components equilibrate, the level of corticosterone in the samples negatively correlates with the level of detectable corticosterone-peroxidase conjugate. After addition of the substrate, the assay signal decreases with increasing amounts of corticosterone. A corticosterone standard is provided to generate a standard curve for the assay and all samples should be read off the standard curve.

# KIT COMPONENTS

Component:	Cat #	K3014-1	K3014-5
Donkey Anti-Sheep IgG Coated 96 Well Plate		1 plate	5 plates
Corticosterone Standard (100,000 pg/mL)		125 µl	625 µl
Corticosterone Antibody		3 ml	13 ml
Corticosterone Conjugate		3 ml	13 ml
1X Assay Buffer		28 ml	55 ml
Dissociation Reagent		1 ml	5 ml
<b>* To be used only with serum and plasma samples</b>			
20X Wash Buffer Concentrate		30 ml	125 ml
TMB Substrate		11 ml	55 ml
Stop Solution		5 ml	25 ml
- 1N hydrochloric acid solution (HCl). <b>CAUSTIC</b>			
Plate Sealer		1 each	5 each

***Store above components at 4°C***

## MATERIALS REQUIRED BUT NOT SUPPLIED

- Supply of distilled or deionized water
- Microplate washer
- Repeater pipet with disposable tips capable of dispensing 25ul, 50ul, and 100ul.
- Colorimetric 96-well microplate reader capable of reading OD at 450 nm with correction between 570 and 590 nm.
- Software for converting raw relative fluorescent unit (FLU) readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting

## PRECAUTIONS

For in vitro research use only. As with all such products, this kit should only be used by qualified personnel who are experienced in routine laboratory procedures and safety practices. Read and understand the complete user manual before attempting to use the product.

**The Stop Solution is 1N hydrochloric acid. Appropriate precautions should be observed when handling this caustic solution. Avoid contact with skin or eyes.**

Wear gloves and laboratory coats when handling materials and, in all cases, please consult your institution's safety procedures for working with hazardous chemicals.

# REAGENT PREPARATION

*Allow the kit reagents to come to room temperature for 30 minutes.*

We recommend that all standards and samples be run in for accurate determination of corticosterone concentrations. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

**The corticosterone stock solution contains an organic solvent. Pre-rinse the pipet tip several times to ensure accurate delivery.**

## Wash Buffer

Dilute 20X Wash Buffer 1:20 by adding one part of the concentrate to nineteen parts of deionized water. Once diluted this is stable at room temperature for 3 months at room temperature.

## Normal Range Standard Preparation

Using the Corticosterone standard stock at (100,000 pg/ml), prepare a series of 7 standards by serial dilution as follows, referring to the table below. Label seven glass test tubes as #1 through #7. Briefly spin vial of standard in a microcentrifuge to ensure contents are at the bottom of the vial.

- Pipet 475  $\mu$ L of Assay Buffer into tube 1 and 250  $\mu$ L into tubes 2 to 7.
- Add 25  $\mu$ L of the corticosterone stock solution to tube 1 and vortex completely.
- Take 250  $\mu$ L of the corticosterone solution in tube 1 and add it to tube 2 and vortex completely.
- Repeat the serial dilutions for tubes 3 through 7. The concentration of corticosterone in tubes 1 through 7 will be 5,000, 2,500, 1,250, 625, 312.5, 156.25, and 78.125 pg/mL.

Reagent	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6	Standard 7
Assay Buffer Volume	475 $\mu$ l	250 $\mu$ l	250 $\mu$ l	250 $\mu$ l	250 $\mu$ l	250 $\mu$ l	250 $\mu$ l
Corticosterone Stock	25 $\mu$ l						
Standard 1		250 $\mu$ l					
Standard 2			250 $\mu$ l				
Standard 3				250 $\mu$ l			
Standard 4					250 $\mu$ l		
Standard 5						250 $\mu$ l	
Standard 6							250 $\mu$ l
Final Concentration (pg/mL)	5,000	2,500	1,250	625	312.5	156.25	78.125

**Use all Standards within 2 hours of preparation.**

## Expanded Range Standard Preparation

Using the Corticosterone standard stock at (100,000 pg/ml), prepare a series of 8 standards by serial dilution as follows, referring to the table below. Label eight glass test tubes as #1 through #8. Briefly spin vial of standard in a microcentrifuge to ensure contents are at the bottom of the vial.

- Pipet 450 µL of Assay Buffer into tube 1 and 250 µL into tubes 2 to 7.
- Add 50 µL of the corticosterone stock solution to tube 1 and vortex completely.
- Take 250 µL of the corticosterone solution in tube 1 and add it to tube 2 and vortex completely.
- Repeat the serial dilutions for tubes 3 through 7. The concentration of corticosterone in tubes 1 through 8 will be 10,000, 5,000, 2,500, 1,250, 625, 312.5, 156.25, and 78.125 pg/mL.

Reagent	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6	Standard 7	Standard 8
Assay Buffer	450 µl	250 µl	250 µl	250 µl	250 µl	250 µl	250 µl	250 µl
Corticosterone Stock	50 µl							
Standard 1		250 µl						
Standard 2			250 µl					
Standard 3				250 µl				
Standard 4					250 µl			
Standard 5						250 µl		
Standard 6							250 µl	
Standard 7								250 µl
Final Concentration (pg/mL)	10,000	5,000	2,500	1,250	625	312.5	156.25	78.125

**Use all Standards within 2 hours of preparation.**

# SAMPLE PREPARATION

This assay has been validated with serum, plasma (EDTA or heparin), cell extract, and dried fecal extract samples. Samples containing visible particulate should be centrifuged prior to using. Moderate to severely hemolyzed samples should not be used in this kit.

**Use samples within 2 hours of dilution.**

## **Serum and Plasma Samples**

Serum and plasma samples should be diluted with an equal volume of Dissociation Reagent. Diluted samples should then be further diluted at least 1:50 with Assay Buffer.

## **Tissue Culture Media**

Corticosterone can be directly measured in Cell Culture supernatant. This assay has been validated with RPMI-1640 medium. The standard curve should be generated using the culture media instead of the Assay Buffer and samples with a high concentration of Corticosterone should be diluted in medium.

## **Dried Fecal Samples**

Weigh out 0.2 g of dried fecal solid. Add 1 mL of Ethanol (or Ethyl Acetate) for every 0.1 gm of solid and vortex for at least 30 minutes. Centrifuge samples at 5,000 rpm for 15 minutes, then transfer supernatant to a clean tube. Evaporate supernatant. Store dried extracted samples at -20°C in a desiccator. Just before assaying, dissolve extracted sample with 100µL ethanol and add at least 400µL Assay Buffer. Vortex well, then allow to rest 5 minutes. Repeat vortex and rest 5 minutes 2-3 times to ensure complete resuspension.

Recommendation: Determine the extraction efficiency by preparing a corticosterone solution of known concentration in the kit assay buffer. Spike one aliquot of your sample with a volume of the corticosterone solution (Control spike) and one aliquot of sample with the same volume of assay buffer (Control sample). Extract both the Control spike and the Control sample with ethanol as described above.

## **Urine Samples**

Urine Samples should be diluted  $\geq 1:20$  with the supplied Assay Buffer prior to running in the assay. Please see our Urinary Creatinine Detection Kit (Cat# K3002-1) for assays to measure urine creatinine which can be used to allow normalization of corticosterone in a random urine specimen.

# ASSAY PROTOCOL

Standards and samples should be run in duplicate

**Be sure to include duplicate Non-Specific Binding (NSB) reaction and blank reactions. The NSB reaction omits the Corticosterone antibody and provides a background signal level for the assay. The blank only includes the Assay Buffer and provides the maximum signal level without corticosterone competition.**

1. Pipet 50  $\mu$ L of samples or standards into each well. Also, pipet 50  $\mu$ L of Assay Buffer into wells for blanks points (Bo or 0 pg/mL) and 75  $\mu$ L of Assay Buffer into the non-specific binding (NSB) wells.
2. Add 25  $\mu$ L of the Corticosterone-Peroxidase Conjugate to each well using a repeater or multichannel pipet.
3. Excluding the NSB wells, add 25  $\mu$ L of the Corticosterone Antibody to each reaction.
4. Gently tap the sides of the plate to ensure adequate mixing, then cover the plate with the plate sealer and shake at room temperature for 1 hour. Note: shaking is necessary to ensure maximum sensitivity.
5. Aspirate the plate and wash 4 times with 300  $\mu$ L Wash Buffer. Tap the plate dry on absorbent towels.
6. Add 100  $\mu$ L of the TMB Substrate to each well.
7. Incubate the plate at room temperature for 30 minutes without shaking.
8. Add 50  $\mu$ L of the Stop Solution to each well.
9. Read the absorbance at 450 nm using a plate reader (the correction should be set at approximate 580 nm).

# CALCULATIONS

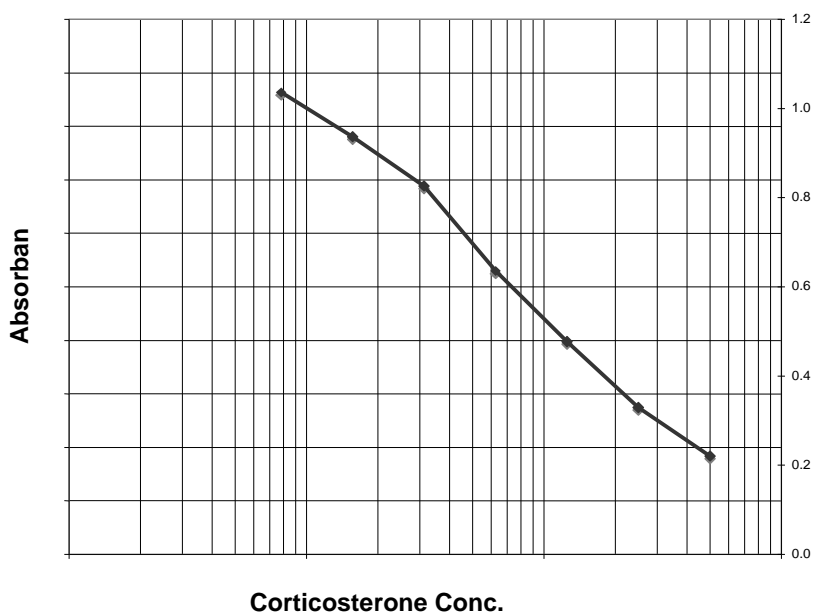
After averaging duplicate absorbance readings, subtract the signals for the NSB to obtain the signal value for each sample and standard. Create a standard curve by fitting the standards to a 4 parameter logistic curve (4PLC). The concentration of corticosterone in each sample is then using the % B/B0 from the curve, then multiply by the dilution value to obtain original concentrations amounts.

**TYPICAL DATA**

Sample	Mean OD	Net OD	% B/B0	Corticosterone Conc. (pg/mL)
NSB	0.047	0	-	-
Standard 1	0.270	0.223	18.4	5,000
Standard 2	0.380	0.333	27.5	2,500
Standard 3	0.529	0.482	39.8	1,250
Standard 4	0.689	0.642	53.0	625
Standard 5	0.882	0.835	68.9	312.5
Standard 6	0.993	0.947	78.1	156.25
Standard 7	1.094	1.047	86.4	78.125
B0	1.259	1.213	100.0	0
Sample 1	0.386	0.339	28.0	2,387.9
Sample 2	1.034	0.987	81.4	127.2

## TYPICAL STANDARD CURVE: EXAMPLE ONLY

Standard curves vary with each assay. Always run your own standard curves for calculation of results; do not use this data



### Notes:

100 pg/ml of Corticosterone is equivalent to 288.6 pM  
Assay sensitivity is 18.6 pg/ml; Limit of detection is 16.9 pg/ml